



Biome boundary maintained by intense belowground resource competition in world's thinnest-rooted plant community

Mingzhen Lu^{a,b,1}, William J. Bond^{c,d,1}, Efrat Sheffer^e, Michael D. Cramer^c, Adam G. West^c, Nicky Allsopp^d, Edmund C. February^c, Samson Chimphango^c, Zeqing Ma^f, Jasper A. Slingsby^{c,d,g}, and Lars O. Hedin^{a,1}

^aDepartment of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544; ^bSanta Fe Institute, Santa Fe, NM 87501; ^cDepartment of Biological Sciences, University of Cape Town, 7701 Cape Town, South Africa; ^dFynbos Node, South African Environmental Observation Network, 7735 Cape Town, South Africa; ^eThe Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, Hebrew University of Jerusalem, Rehovot 76100, Israel; ^fQianyanzhou Ecological Research Station, Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China; and ^gCentre for Statistics in Ecology, the Environment and Conservation, University of Cape Town, 7701 Cape Town, South Africa

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Recent findings point to plant root traits as potentially important for shaping the boundaries of biomes and for maintaining the plant communities within. We examined two hypotheses: 1) Thin-rooted plant strategies might be favored in biomes with low soil resources; and 2) these strategies may act, along with fire, to maintain the sharp boundary between the Fynbos and Afrotropical Forest biomes in South Africa. These biomes differ in biodiversity, plant traits, and physiognomy, yet exist as alternative stable states on the same geological substrate and in the same climate conditions. We conducted a 4-y field experiment to examine the ability of Forest species to invade the Fynbos as a function of growth-limiting nutrients and belowground plant–plant competition. Our results support both hypotheses: First, we found marked biome differences in root traits, with Fynbos species exhibiting the thinnest roots reported from any biome worldwide. Second, our field manipulation demonstrated that intense belowground competition inhibits the ability of Forest species to invade Fynbos. Nitrogen was unexpectedly the resource that determined competitive outcome, despite the long-standing expectation that Fynbos is severely phosphorus constrained. These findings identify a trait-by-resource feedback mechanism, in which most species possess adaptive traits that modify soil resources in favor of their own survival while deterring invading species. Our findings challenge the long-held notion that biome boundaries depend primarily on external abiotic constraints and, instead, identify an internal biotic mechanism—a selective feedback among traits, plant–plant competition, and ecosystem conditions—that, along with contrasting fire regime, can act to maintain biome boundaries.

biome boundary | resource competition | root traits | nitrogen | trait-by-resource feedback

Recent findings (1) have demonstrated striking differences in plant rooting strategies across biomes worldwide, spawning the hypothesis that belowground competition for soil resources may be critical for maintaining biome boundaries (1, 2). This idea differs fundamentally from the historical notion that biomes primarily are delineated by extrinsic abiotic factors such as climate, geological parent material, or topography (3–8), or the more recent recognition that aboveground plant adaptations can promote fire-determined plant communities (9, 10).

Belowground competition introduces a biotic mechanism that is intrinsic to the plant community, emerges from plant–plant contest for resources, and may help explain the puzzling observation that biome boundaries can persist independent of climate–geological factors (4, 10).

Of central importance is Ma et al.'s (1) recent observation that root traits that are associated with resource uptake appear to differ across biomes with differing soil resource dynamics.

Specifically, Ma et al. hypothesized that thin-rooted plant strategies may be favored in biomes with permanently or seasonally low soil resources. They reasoned that, in those conditions, natural selection would favor absorptive roots [i.e., first-order roots (1, 11)] with low diameter and high specific root length (i.e., root length per unit photosynthetic carbon invested), which, in turn, are traits that allow high root surface area and efficient exploration of resource-poor soils. Conversely, thick roots and low specific root length may remain competitive traits in biomes with abundant soil resources, despite reduced root surface area and less efficient soil exploration.

Here we test Ma et al.'s hypothesis (1) using a unique study of root traits and plant–plant resource competition across the boundary of two distinct biomes within the Cape Floristic Region of South Africa: Fynbos and Afrotropical Forest. We show in Fig. 1 and Table 1 that—despite co-occurring across

Significance

The distribution and stability of biomes are critical for understanding, modeling, and managing the land biosphere. While studies have emphasized abiotic factors such as climate, geology, or fire regimes, we here identify a biological mechanism—plant–plant competition for belowground resources—as critical for maintaining the boundary between the Fynbos and Afrotropical Forest biomes in South Africa. We demonstrate an apparent general mechanism in which local competition triggers a biome-scale feedback between plant traits and soil resources, which, in turn, stabilizes the biome boundary by allowing plants to maintain their own preferred soil conditions. Our findings are of general importance for understanding the organization of biodiversity across landscapes, for managing alien plant invasions, and for modeling the future of biome boundaries.

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¹To whom correspondence may be addressed. Email: mingzhen.lu89@gmail.com, william.bond@uct.ac.za, or lhedin@princeton.edu.

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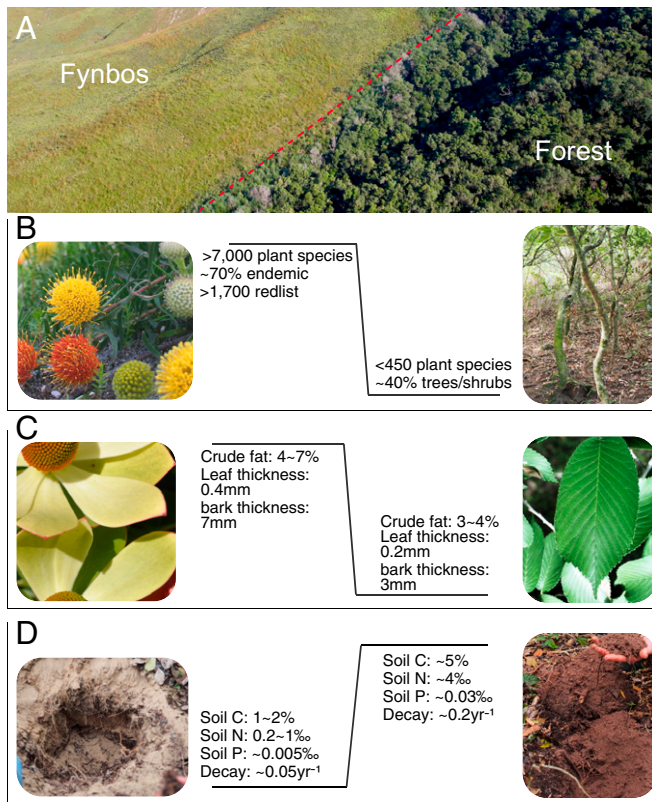


Fig. 1. Sharp differences in biodiversity, aboveground plant traits, and ecosystem properties across the South African Fynbos–Forest boundary. (A) Two neighboring biomes of the Cape Floristic Region—the Fynbos (62) and the Afrotemperate Forest (63)—form a sharp boundary despite perching on the same geological parent material (39). (B) Biodiversity: The hyperdiverse Fynbos harbors >7,000 plant species, of which the majority are endemic to South Africa (64). The Afrotemperate Forest, on the other hand, contains >450 species with less endemism (63). (C) Aboveground plant traits: Fynbos species generally possess thick and small leaves with a high carbon-to-nitrogen (C:N) ratio while Afrotemperate Forest species display thinner and larger leaves with a lower C:N ratio. In addition, Fynbos plant species possess traits that either enhance (e.g., waxes) or resist (e.g., thick bark) fire. For example, Fynbos vegetation contains high concentrations of flammable organic compounds (e.g., crude fat content) that can facilitate very hot fires (65). In contrast, Afrotemperate Forest species tend to be sensitive to fire and possess traits that suppress fire (e.g., high water content). (D) Ecosystem properties: Fynbos soils are exceedingly poor in soil carbon, nitrogen, and phosphorus contents. In contrast, the Afrotemperate Forest soil is characterized by a developed layer rich in carbon, nitrogen, and phosphorus, which facilitates active cycling of nutrients between plant and soil pools (Table 1). Moreover, Fynbos litter decays about five times slower when placed in the Fynbos biome compared with forest litter in the Afrotemperate Forest biome (66, 67).

the same climate–geological conditions—Fynbos and Afrotemperate Forest differ dramatically in the abundance, seasonality, and recycling of soil resources. Specifically, the Fynbos biome is characterized by some of the lowest levels of soil phosphorus and nitrogen of any biome worldwide (12, 13), by slow decomposition and nutrient recycling (14), and by low stores of soil organic matter (15). In contrast, the Afrotemperate Forest biome is defined by a substantial accumulation of soil organic matter and organic-bound nutrients, which, in turn, supports high rates of plant–soil–nutrient recycling. Based on Ma et al.’s hypothesis, we would expect that these sharp differences in soil resource conditions would result in divergent belowground root traits across the biome boundary.

We further hypothesize that these differences in root traits, when combined with plant–plant competition for belowground

resources, may offer a mechanism that acts to reinforce the boundary between the Fynbos and Afrotemperate Forest biomes. Central to such a mechanism is the emergence of a trait-by-resource feedback (2, 16), in which a plant species possesses traits that can impact the local conditions and recycling of soil resources. A biotic feedback can emerge if, in turn, the resulting resource regime acts to promote the resident plant species and/or to prohibit the invasion by nonresident species. In this way, a trait-by-resource feedback can in theory (16) maintain a biome boundary independent of differences in geological parent material or climate factors.

An important (but not sufficient) part of this trait-by-resource feedback is that plant root traits must be systematically coupled to plant characteristics that can influence resource dynamics at the ecosystem scale. A notable example is the Fynbos biome (Fig. 1), in which plant species possess traits that promote fires at return times of ~10 to 40+ y (17, 18). These fires, in turn, are hot enough to induce severely nutrient-poor soil conditions by volatilizing soil and plant organic nitrogen (19, 20) and by increasing the likelihood that phosphorus can leach from the soil profile following rain events (21). However, the feedback can only function if aboveground fire-adapted traits are systematically coupled with belowground traits that allow Fynbos plant species to outcompete any invading plants from the nearby Afrotemperate Forest. Conversely, the Afrotemperate Forest plant community depends on conditions that favor the significant accumulation of an organic soil nutrient pool (Fig. 1), which, in turn, can facilitate the active cycling of nitrogen and phosphorus between the plant and soil components of the ecosystem.

We experimentally tested the belowground component of this Fynbos trait-by-resource feedback idea, using a 4-y field experiment in which we manipulated 1) the supply of the potentially growth-limiting resources nitrogen and phosphorus, and 2) the ability of plants to compete for nitrogen and phosphorus belowground. Specifically, we asked whether Afrotemperate Forest tree species could successfully invade the Fynbos plant community, across differing conditions of soil resources and belowground competition. In the field, we established a full factorial manipulation of nitrogen and phosphorus across 40 plots in two separate locations within the native Fynbos plant community (*Materials and Methods* and *SI Appendix*, Fig. S2). We transplanted forest tree seedlings into all experimental plots and evaluated their ability to grow across the different soil nutrient and competition scenarios (*SI Appendix*, Fig. S3).

Overall, our project was designed to evaluate whether Fynbos plants possess root traits that are consistent with a high capacity to compete for scarce nutrients and, in turn, whether these traits translate into the ability to outcompete plant species that seek to invade the Fynbos plant community—as predicted by the trait-by-resource feedback mechanism.

Results

World’s Thinnest-Rooted Biome. Our results show that the nutrient-poor and seasonal conditions of the Fynbos biome were associated with exceedingly thin-rooted plant species, and with root traits that are consistent with natural selection for efficient nutrient acquisition. First, the diameter of first-order absorptive roots differed dramatically across 13 families and 15 genera of representative Fynbos and Afrotemperate Forest species (Fig. 2A, brown vs. green points). Specifically, we found statistically clear separations of root diameter when analyzed across biomes alone (Fig. 2A, density plot; $P < 0.001$, linear model; $n = 810$) or across biomes when considering individual species as a random effect (Fig. 2A, boxplot; $P < 0.001$).

We next compared our species-specific observations against a dataset from major biomes worldwide (1). This comparison

Table 1. Comparison of neighboring Fynbos and Afrotemperate Forest

Properties and traits	Fynbos	Afrotemperate Forest
Ecosystem properties		
Fire return interval, y	12~20*	n.a.
Soil carbon, mg/g	23.5(5, 4.9), [†] 9.2(1.4) [†]	49.3(5, 4.4) [†]
Soil nitrogen, mg/g	1.07(5, 0.29), [†] 0.15(0.01), [†] 1.3(0.6) [§]	3.24(5, 0.26), [†] 3.9(0.8) [§]
Soil phosphorus, mg/kg	6.8(5, 2.8), [†] 4.8(0.9) [§]	28.4(5, 2.5), 22.5(8.6) [§]
Litter decomposition rate, y ⁻¹	0.07, [¶] 0.05 [#]	0.24
Litter half-life time, y	10, [¶] 14 [#]	2.9
Canopy cover, %	20(360, 0.76)**	81(9, 0.03)**
Aboveground plant traits		
Maximal height, m	0.84(309, 0.05)**	17(26, 0.92)**
Leaf thickness, mm	0.44(309, 0.15)**	0.19(143, 0.005)**
Leaf size, cm ²	7.5(309, 1.7)**	20.4(143, 1.7)**
Specific leaf area, cm ² /g	60(309, 2.2)**	105(143, 8.1)**
A _{max} , μmol CO ₂ ·m ⁻² ·s ⁻¹	18(16–20) ^{††}	8.6(7.5–9.8) ^{††}
Crude fat content, %	4.3–6.7 ^{††}	2.6–4.0 ^{††}
Fuel moisture content, %	86–15 ^{††}	139–229 ^{††}
C:N ratio	66, ^{§§} 95 ^{§§}	18 ^{¶¶}
Bark thickness, mm	7.2 [#]	~3

Though sharing similar climatic and geological conditions, the Fynbos and Afrotemperate Forest biomes differ in their ecosystem properties and plant traits. Values in parentheses identify the sample size and SE from our study. n.a., not applicable.

*Estimate from ref. 68

[†]Soil total carbon, total nitrogen, and available phosphorus were derived from five pairs of Forest–Fynbos sites immediately neighboring each other at the Orange Kloof site in the Table Mountain National Park of Cape Town (*Materials and Methods*).

[‡]Zero- to 10-cm soil of sandplain lowland Fynbos of Cape Province (69).

[§]Direct comparison of neighboring Forest and Fynbos across four sites in Swartboskloof (42).

[¶]Based on a 3-y field incubation study using the common species *Leucospermum parile* (70).

[#]Based on a 2.5-y field incubation study using the common species *P. repens* (71).

^{||}Based on the evergreen tree *Pterocelastrus tricuspidatus* (50).

**Plant traits compiled by our group.

^{††}Mean (95% CI) digitizer from figure 1a of ref. 72 and rounded to double significant digits. Five Forest species (*D. whyteana*, *K. africana*, *Olea capensis*, *Olea europaea*, and *Rapanea melanophloeos*) and four Fynbos species (*Berzelia lanuginosa*, *Erica versicolor*, *Phyllica ericoides*, and *Searsia lucida*) were used.

^{‡‡}Crude oil includes oils, fats, waxes, and terpenes that are extracted using the Soxhlet extraction approach (65). For both crude fat content and fuel moisture content, we derived the Fynbos value from the simple mean of the dominant Fynbos species (*P. neriifolia*, *Cliffortia cuneata*, *B. nodiflora*, and *Erica plukenetii*) and derived the Forest values from six Forest species (*C. capensis*, *Ilex mitis*, *K. africana*, *Maytenus oleoides*, *Brachylaena neriifolia*, and *Brabejum stellatifolium*) (65).

^{§§}The first value is derived from table 3 of ref. 73 using the simple mean of four Fynbos elements (proteoid, ericoid, restioid, and other sclerophylls) across coastal and mountain habitats. The second value is the average C:N ratio of the dominant canopy proteoid species.

^{¶¶}The simple mean leaf nitrogen concentration of 107 Afrotemperate Forest species across South Africa from ref. 74 is first calculated (25.95 mg/g). Assuming the average carbon concentration is equal to the global average leaf carbon content [476 mg/g (75)], the average C:N ratio is derived.

[#]Bark thickness data of Fynbos species standardized at 5-cm trunk diameter are from woody *Protea* species that are resistant to fire (76). Restioids, ericoids, grass growth forms, and non–fire-resistant *Protea* species are pyrophilic. (Forest bark thickness data of Afromontane Forest from Knysna area are from unpublished data.)

revealed that the Fynbos biome stands out as possessing the world’s thinnest-rooted plant species measured so far (Fig. 2B, brown points; $\alpha = 0.05$, pairwise contrast analysis), with median root diameter two to four times smaller than any other biome. We also discovered that nonforested biomes with permanently and/or seasonally low soil resources—Fynbos, Grassland, Desert, and Mediterranean—occupy the lower end of the diameter spectrum, but also that Fynbos roots are significantly thinner than all other seasonal (and nonseasonal) biomes ($\alpha = 0.05$, pairwise contrast analysis). In contrast, the root diameters of our Afrotemperate Forest species (Fig. 2B, green points) did not differ significantly from other biomes characterized by higher and/or seasonally sustained resource supplies—Tropical, Subtropical, Temperate, or Boreal forests.

These broad trends translate into order-of-magnitude differences in specific root length across biomes (Fig. 2C), a measure of the length of soil explored per unit of plant investment in root biomass. We derived specific root length based on the median global tissue density of first-order roots (1), as detailed in *Materials and Methods*. The median specific root length of Fynbos species was 10 times greater than that of Afrotemperate Forest species (587 vs. 57.8 m/g, respectively; $P < 0.001$, Welch

t test), indicating that Fynbos species are substantially more efficient at exploring soil per unit of photosynthetic carbon invested. The specific root length of our Fynbos species was similar to observations from a group of seasonal nonforested biomes (Desert, Mediterranean, and Grassland), but 6 of 14 Fynbos species exceeded all observations from any other biome worldwide (Fig. 2C). In contrast, a second group of forested biomes (Tropical, Subtropical, Temperate, Afrotemperate, and Boreal forests) displayed significantly lower specific root lengths ($P < 0.001$, mixed-effects model).

We next considered root branching intensity—the number of first-order root branches per length of second-order roots—as a potentially important trait for plant nutrient acquisition (22). We found significantly higher branching intensity in the Fynbos than in the Afrotemperate Forest species (Fig. 2D; $P = 0.017$, Student *t* test), despite a high degree of interspecific variation within each biome. Data from other biomes are sparse, but a comparison against a dataset from the Subtropical forest biome (22) suggests that both Fynbos and Afrotemperate Forest species feature higher branching intensities (Fig. 2D; $P < 0.001$, Student *t* test).

Taken together, these results indicate that key resource-related root traits of Fynbos plant species are uniquely different

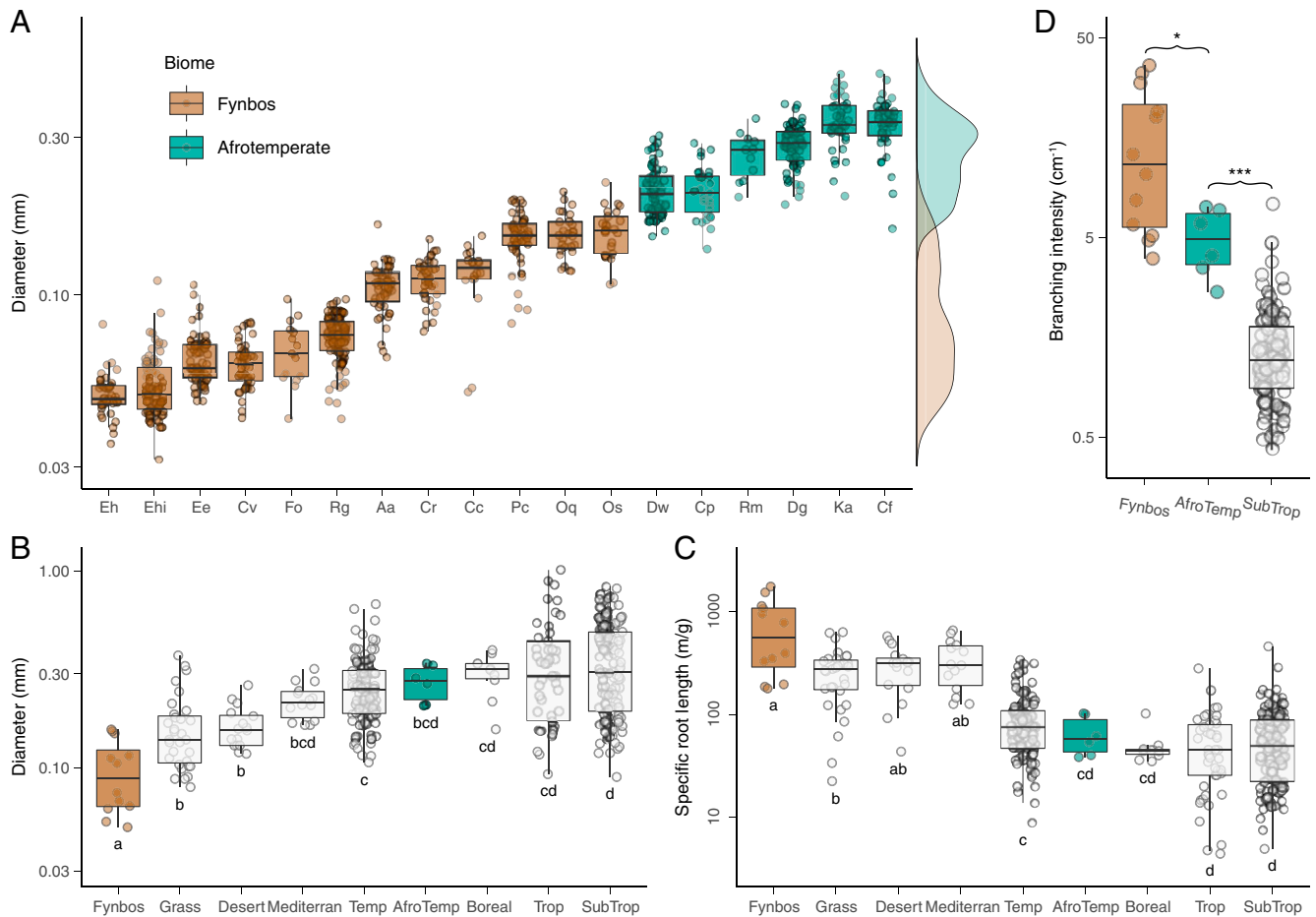


Fig. 2. Comparison of plant root traits across species and biomes. Across all panels, we graphically rank species/biomes based on the median, with each dot representing either the mean value of one root segment (A) or the mean of one species (B–D). (A) Fynbos species generally have thinner first-order roots than neighboring Forest species, with little overlap of distribution even at the root segment level. Species names are abbreviated for display and are detailed in *SI Appendix, Table S1*. (B) Fynbos, on average, has the thinnest first-order roots among all terrestrial biomes measured, even significantly thinner than other seasonal biomes. Afrotemperate Forest, on the other hand, is very similar to other forest biomes. (C) Fynbos, on average, has the highest specific root length (first-order roots) across all terrestrial biomes measured, while Afrotemperate Forest is very similar to other forest biomes (e.g., Temperate and Subtropical forests). To calculate specific root length for Fynbos and Afrotemperate Forest species, we assumed that root tissue density of all species equals 0.29 g/cm^3 (*Materials and Methods*). In B and C, the letters “a,” “b,” and so forth denote significant differences between categories ($\alpha = 0.05$). Previously published data from Ma et al. are denoted as white-filled circles and boxplots (1). (D) Fynbos roots have higher branching intensities than the roots in the Afrotemperate Forest and Subtropical Forest biomes ($*P < 0.05$, $***P < 0.001$). Previously published data from Kong et al. are indicated as white-filled circles and boxplots (22).

from the nearby Afrotemperate Forest biome and from other biomes worldwide. Fynbos species possess thinner roots, higher specific root length, and generally higher root branching intensity.

Intense Belowground Competition Inhibits Forest Establishment. We next asked whether the nutrient-poor conditions (Fig. 1 and Table 1) and the unique root traits (Fig. 2) combine to protect the Fynbos plant community from invasion by plants from the nearby Afrotemperate Forest biome.

We transplanted tree seedlings of three common Afrotemperate Forest species into a full factorial 4-y manipulation of phosphorus and nitrogen within the native Fynbos plant community. Six tree seedlings were planted in each of forty 4×2.5 -m plots distributed across two replicate Fynbos communities (*SI Appendix, Fig. S2*). Within each plot, we used a root-exclusion technique (23) to manipulate belowground competition between individual tree seedlings and the native Fynbos community (*Materials and Methods* and *SI Appendix, Fig. S3*).

Nutrient conditions played a critical role in determining the ability of invading tree seedlings to grow within the Fynbos community. Nitrogen addition alone increased the growth rate of trees by more than fivefold (Fig. 3A; $P = 0.013$, mixed-effects model). In contrast, phosphorus addition had no statistically detectable effect on tree growth, despite the severely phosphorus-poor conditions of the Fynbos soil ($P = 0.78$, mixed-effects model). Finally, the combined addition of nitrogen and phosphorus did not produce a growth response beyond what would be expected from the addition of nitrogen alone ($P = 0.87$ for phosphorus-by-nitrogen interaction, mixed-effects model).

Our experiment also demonstrated that the exclusion of belowground root competition triggered a fivefold increase in tree growth rates (Fig. 3B; $P = 0.016$, mixed-effects model). The magnitude of this growth response was similar to that of adding nitrogen (Fig. 3A), indicating that both treatments (competition exclusion and nitrogen addition) released growth limitation to a similar extent. These results point to nitrogen alone as the central resource for competition between the native Fynbos and the invading Afrotemperate Forest plants.

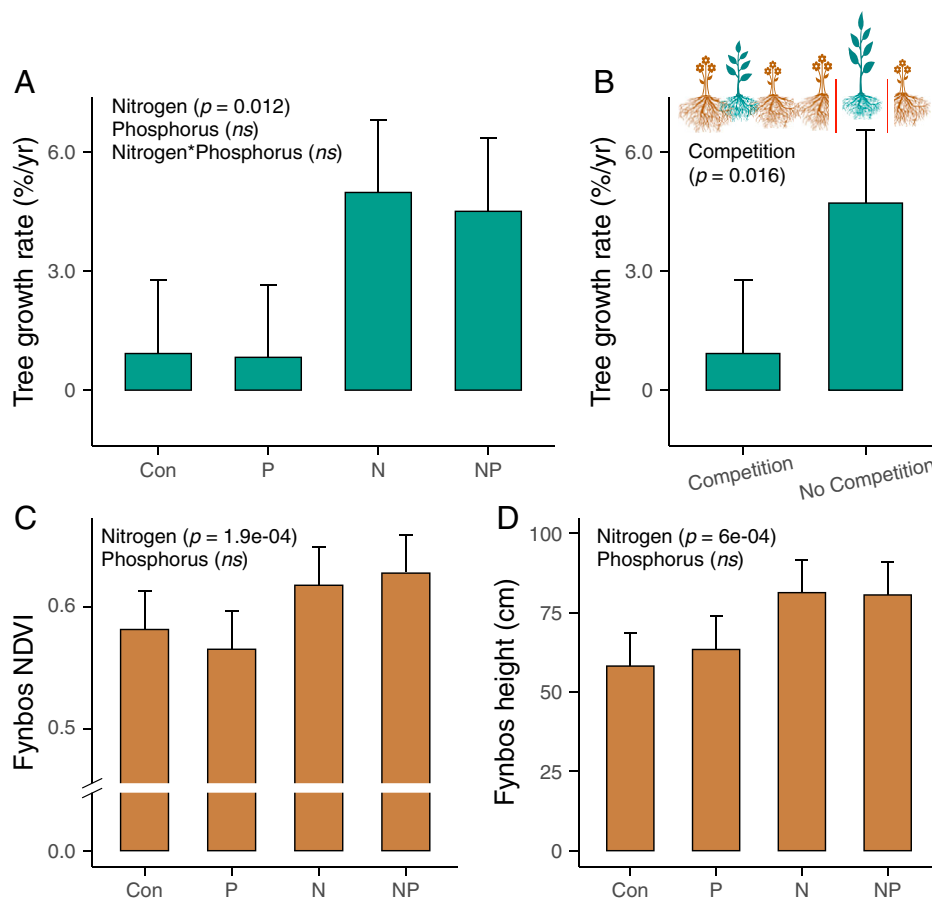


Fig. 3. Manipulation of nutrients and belowground competition impacts the growth of transplanted Forest tree species (A and B) and native Fynbos vegetation (C and D). (A) Relative growth rate of transplanted tree seedlings (basal diameter-based) is not statistically different from zero ($0.9\% \cdot y^{-1}$, $P = 0.56$). The addition of nitrogen additionally enhanced growth rate by $3.9\% \cdot y^{-1}$ ($P = 0.012$, linear mixed-effects model) independent of competition exclusion. We did not find significant influence of phosphorus addition on Forest tree growth ($P = 0.78$, linear mixed-effects model). We derived these results based on 564 observations across 94 individual tree seedlings using a linear mixed-effects model (*Materials and Methods*). Con, control; ns, not significant. (B) Alleviating belowground competition increases the relative growth rate of the transplanted Forest seedling by $3.8\% \cdot y^{-1}$ ($P = 0.016$), independent of nutrient fertilization. Tree seedlings are labeled green while Fynbos plants are labeled brown in the illustration, with two red vertical bars denoting a 30-cm-high, 20-cm-wide PVC collar. (C) Fynbos plots that received nitrogen addition had significantly higher NDVI (as a proxy for living vegetation biomass) ($P < 0.001$). We did not find significant influence of phosphorus on Fynbos NDVI. The results are based on reoccurring drone-based observation (~2 and 4 y after fire) of 40 plots using a linear mixed-effects model (*Materials and Methods*). The results are based on the average of two time periods. The brown color indicates results for Fynbos responses, consistent with the Fynbos color in Fig. 2. Notice that we implemented a break on the y axis to aid the visualization of the nutrient treatment effects, due to the high background value of NDVI. (D) Fynbos plots that received nitrogen addition have significantly higher vegetation height ($P < 0.001$). The results are based on reoccurring in situ observation (~2 and 4 y after fire) of 40 plots using a linear mixed-effects model (*Materials and Methods*). Error bars represent SE of the modeled means.

Finally, we examined the response of the native Fynbos plant community to our nutrient manipulation. As observed for the tree seedlings discussed above, only the addition of nitrogen caused increased growth, as quantified by net change in normalized difference vegetation index (NDVI) (Fig. 3C) and in plant height (Fig. 3D) compared with the control treatment. Phosphorus—alone or combined with nitrogen—stimulated no statistically significant additional growth, despite the phosphorus-poor soils (12) and the well-documented adaptations of Fynbos plant species to low phosphorus conditions (24, 25).

Fynbos Species Outgrow Forest Seedlings in Height Competition. Our experiment also demonstrated that height growth of the native Fynbos plant community exceeded that of the transplanted Afrotropical Forest tree seedlings over the 4-y experimental period (Fig. 4). In contrast, the height of Afrotropical Forest seedlings (no nitrogen fertilization) changed little over the course of our experiment, highlighting the critical role of belowground nutrient competition in maintaining the carbon balance of the tree seedlings (Fig. 3A and B).

Discussion

Most broadly, our study adds to the growing recognition that belowground plant traits can feed back upon plant community properties, with significant implications for the structure and dynamics of land ecosystems (2, 26).

Fynbos Plants Feature Acquisitive Root Traits. Our results demonstrate that plant species from the Fynbos biome possess root traits that favor competition at low soil resource levels. First, Fynbos species produced substantially higher numbers of root tips per length of parent root when compared with Afrotropical Forest species. This means that species in the Fynbos community can more efficiently explore microsize nutrient patches in the soil, gather information about the soil matrix, and change foraging directions (22, 27). As a result, the plants can better compete for the exceedingly low quantities of nitrogen and phosphorus present in the Fynbos soil.

Perhaps more importantly, the roots of plant species from the Fynbos community were dramatically thinner than the roots

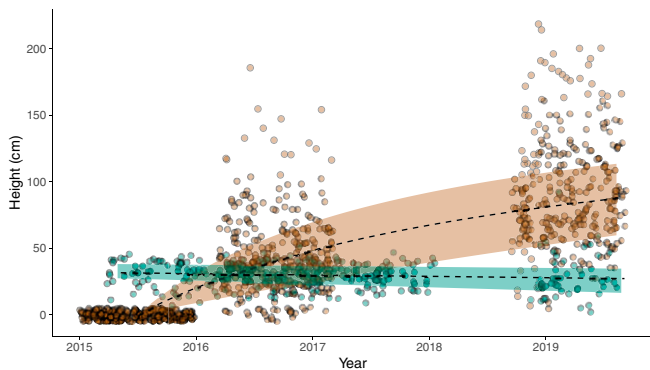


Fig. 4. Height competition between native plants from the Fynbos community (brown) and transplanted trees from the Afrotropical Forest community (green). During the same 4-y period following a hot fire, plants in the Fynbos community outgrow the transplanted trees in height. Each green dot represents an individual Forest tree seedling growing without nitrogen fertilization, while each brown dot represents one measurement of the Fynbos community at a given locus in our study plots. For visual guidance, we fitted a Michaelis–Menten function to the mean plant height of the Fynbos vegetation (dashed lines with 75 and 25% quantile boundaries), but the exact functional form that best captures the biology is not central to the present study. For the Afrotropical Forest trees, we fitted a linear relationship to the mean tree height over time, and characterized upper and lower boundaries using the 75 and 25% quantiles. We introduced horizontal (180-d) and vertical (5-cm) jitter to better visualize points that overlap during the individual sample events.

of species from the nearby Afrotropical Forest. In fact, comparison with a global dataset (Fig. 2B) showed that the first-order roots of our Fynbos species were thinner than the species from any other biome that thus far has been measured worldwide. In contrast, roots from our Afrotropical species did not differ significantly from forested biomes worldwide.

The thinness of Fynbos roots translates into high specific root length, which, in turn, offers a strategy by which plants can leverage photosynthetic carbon to efficiently explore the soil volume. The resulting difference between Fynbos and Afrotropical Forest species can be astounding: For example, for each gram of biomass invested in absorptive root tissue, the common Fynbos species *Erica hispidula* (*Eh* in *SI Appendix*, Table S1) produces roots that are more than 15 soccer or football fields longer (1,752 vs. 40 m) than the common Afrotropical Forest species *Chionanthus foveolatus* (*Eh* vs. *Cf* in *SI Appendix*, Fig. S1A).

Overall, these findings are consistent with Ma et al.’s hypothesis (1) that natural selection ought to favor thin first-order roots and high specific root length in biomes that are characterized by low or seasonally low levels of soil nutrients. Indeed, Fynbos is one of the most nutrient-poor and seasonal biomes in the world, and our study (Fig. 2) indicates that root traits of Fynbos plant species stand out compared with other biomes worldwide.

The Fynbos and Mediterranean biomes experience similar climate and seasonality, but the roots of our Fynbos species were significantly thinner (Fig. 2B). It would appear likely that the thin rootedness of these Fynbos plant species has evolved as a result of plant–plant competition for quantities of soil nitrogen and phosphorus that are scarcer than what can be found in the Mediterranean biome (13, 28). Soils in southwestern Australia—that harbor a plant community (i.e., Kwongan) similar to Fynbos—are comparably nutrient-poor (28). While we are aware of no published dataset of first-order roots from this plant community (but see refs. 29 and 30 for an account of root traits/strategies in Kwongan), future studies in Kwongan or other nutrient-poor systems (e.g., sandplain communities of Western Australia, Succulent Karoo, Tundra, etc.) could constitute tests of the generality of our findings.

Our results suggest that thin rootedness should be added to other traits that favor growth in nutrient-poor soil conditions: possession of cluster roots [for facilitating phosphorus uptake (25)], ability to fix nitrogen [nitrogen uptake (31, 32)], association with mycorrhizal symbionts (24), elevation of biomass C:N and C:P ratios (33, 34), and extension of leaf longevity (35). For mycorrhizal plant species, the fineness of first-order roots needs to be considered alongside the extent of reliance on mycorrhizae: Thin-rooted species appear to depend less on mycorrhizae than thick-rooted species (1). Thin rootedness might therefore help explain why Fynbos species in general rely less on mycorrhizae than plants in many other regions (24).

Taken together, our results indicate that Fynbos plant species are uniquely adapted by possessing root traits that make them excellent competitors for scarce soil nutrients when compared with forest-derived plant species. We next discuss our experimental test of this idea, in which we evaluated the ability of Fynbos vs. Afrotropical Forest plant species to compete in situ for soil nitrogen and phosphorus.

Fynbos Species Inhibit the Growth of Forest Species, but Only in Conditions of Low Soil Resources. Our nutrient manipulation allowed us to distinguish three properties that defined the competition between Fynbos and Afrotropical Forest plant species (Fig. 3). First, thick-rooted Forest species grew exceedingly slowly in the native Fynbos soil in conditions of competition with thin-rooted Fynbos plant species. Second, Forest species grew well when supplied with nitrogen. Third, Forest species also grew well when we removed belowground competition by the native Fynbos plant community.

Our results thus identify the growth process as the key step that limits tree invasion into the Fynbos, considering that earlier studies have shown that neither seed dispersal (36) nor germination processes (37) limit tree invasion into the Fynbos. More specifically, the results demonstrate intense belowground competition in native Fynbos soils, and suggest that competition for soil nitrogen can keep invading Forest species from expressing positive growth, and thus from establishing at the biome boundary. This competitive success of the Fynbos plant community is correlated with belowground traits that confer efficient nutrient uptake as well as aboveground traits that promote fire-induced losses of nitrogen (and phosphorus) from the ecosystem. Additional traits that permit Fynbos species to persist in the resulting low-nutrient environment include extended leaf longevity and low leaf nutrient content (Fig. 1).

In contrast, our Afrotropical Forest species carry traits for success in conditions of a well-developed plant–soil nutrient cycle, and absent recurring fire (Table 1). High input of nutrient-rich leaf litter and lack of hot fires enable the accumulation of substantial quantities of organic carbon and organic-bound nutrients in Forest soils (Fig. 1 and Table 1). As a result, Forest plant species have evolved traits that favor competition in conditions of a well-developed plant–soil nutrient cycle, a sustained supply of mineralized nitrogen (from soil organic pools), and the absence of recurring fire.

Our findings differ from the traditional notion that plant biome boundaries are governed by abiotic climate/soil factors or by aboveground plant–fire dynamics (3, 6, 9, 15, 38, 39). Instead, our results suggest the existence of a trait-by-resource feedback, in which root traits and plant–plant resource competition are critical components for determining the ability of plant species to successfully cross a biome boundary. Both abiotic soil conditions and fire are likely contributors to defining the Fynbos–Forest biome boundary, but our findings point to belowground biotic interactions as central for creating an “invasion bottleneck” that acts to stabilize the biome boundary. This bottleneck stunts the ability of trees to grow, and therefore

increases the likelihood that, over time, they will be eliminated by either plant–plant competition or recurring fire.

Nitrogen—but Not Phosphorus—is the Critical Resource. Our results identify nitrogen as the key limiting resource, but also that phosphorus did not significantly influence plant–plant competition—alone or in combination with nitrogen. This lack of a phosphorus effect is surprising given the exceedingly low phosphorus content of the Table Mountain Sandstone parent material (12), the low phosphorus concentrations of leaves of plant species (34), and the abundance of plant adaptations to phosphorus-poor conditions across Fynbos and the analogous Kwongan plant community in Australia [e.g., cluster roots, non-phospholipids in photosynthesis (40, 41)]. In fact, the Fynbos biome is generally considered one of the most phosphorus-limited plant communities worldwide (34, 42), though previous field experiments have raised the possibility that nitrogen may impose the stronger constraint on the plant community (43, 44).

The apparent lack of phosphorus influence may suggest that the numerous adaptations to phosphorus-poor soils that have been demonstrated in plant species from the Fynbos and similar plant communities (40, 41) may instead allow plants to overcome phosphorus limitation. Phosphorus is retained as ash following hot Fynbos fires (21), while nitrogen is efficiently volatilized and thus permanently removed from the ecosystem (19, 20). It is therefore plausible that—over time—plants can recover and recycle phosphorus but not nitrogen in the fire-prone Fynbos biome.

It is not clear why the process of symbiotic nitrogen fixation in native Fynbos communities is not strong enough to enrich soil nitrogen over time. This is particularly puzzling given that the nitrogen-fixing Fabaceae is the second-most diverse family in the Fynbos biome (45). Possible explanations include that phosphorus and/or molybdenum [which are both needed in the fixation process (46–48)] may be too low to sustain high fixation rates, or that fire excludes tree species that may possess high fixation capacity. Empirical observations from native Fabaceae species in the Fynbos biome seem to support both mechanisms: Populations appear to peak in abundance when available phosphorus is temporarily high following fire (21).

Invasion by Non-Fynbos Species. Two observations support the idea that scarce nutrients and recurring fire can shield the Fynbos from invasion by non-Fynbos plant species. First, native Forest and Fynbos species do not appear to differ in germination requirements, but seedlings of Forest species can establish in Fynbos patches that contain especially nutrient-rich soil (37). In such soils, and absent fire, we expect that Forest seedlings would grow more rapidly than what we observed (Fig. 4), and that Forest species eventually can outshade the Fynbos community.

Second, Fynbos is vulnerable to invasion by some alien plant species that are preadapted to both exceedingly low soil nutrients and recurring fire. One example is *Acacia* species (49) that can fix atmospheric nitrogen and that have been brought in from regions of Australia in which soil phosphorus (and possibly molybdenum) is scarce and fire is frequent (50). A second example is Californian *Pinus* species (51) that are adapted to fire and that rely on ectomycorrhizal fungi for accessing organic (instead of inorganic) forms of soil nutrients. The observation that no native Fynbos species have evolved symbiotic associations with ectomycorrhizal fungi (24) implies a possible historical–evolutionary constraint on the ability of the Fynbos biome to withstand alien plant invasions.

“Keep It Miserable” as an Evolved Plant Strategy in a Trait-by-Resource Feedback. Overall, our findings offer a general mechanism for how plants from diverse lineages can act together to modify the Fynbos soil, and thereby promote their preferred

growing conditions. Central to this phenomenon is a trait-by-resource feedback, in which below- and aboveground plant traits are coupled in a manner that promotes losses of nutrients in general, and of nitrogen in particular. In turn, these losses maintain the Fynbos soils in a state of “nutritional misery” for non-Fynbos species by minimizing the local concentration and recycling of nitrogen in particular (see ref. 16 for theoretical derivation).

On the one hand, Fynbos species possess hyperthin roots and other adaptations that allow them to compete well in nutrient-poor environments (Figs. 2 and 3). On the other hand, Fynbos species also possess aboveground traits that allow them to both promote and survive recurring fire (52) (Table 1). In theory, this trait combination can allow for a selective feedback between plant characteristics and ecosystem conditions that disfavors the invasion by species that are not preadapted to both low nutrients and hot fire. The resulting Keep It Miserable strategy differs from traditional game-theoretic strategies (e.g., Hawk, Dove, or Cheater) by relying on an ecosystem-scale resource feedback (16).

Crucially, this trait-by-resource feedback would be evolutionarily stable against invasion by alternative strategies, unless these include adaptations that simultaneously solve the challenges imposed by scarce soil resources and hot fire. Over evolutionary time, it may be difficult for Forest species to solve both challenges since 1) fire does not act as a common selective agent in the Forest, and 2) supply of nitrogen (and other nutrients) from sustained mineralization of soil organic matter does not select for root traits that favor the ability of plants to compete effectively for nutrients at low soil concentrations.

In total, our findings raise the possibility that a lethal combination of below- and aboveground trait strategies, such as Keep It Miserable, may be critical to defining and maintaining boundaries between plant communities at the biome scale.

Materials and Methods

Belowground Trait Sampling and Characterization. To compare the belowground root traits between the Fynbos and Afrotropical Forests, we collected fine root samples from common species in natural Fynbos and Forest communities in the Jonkershoek Nature Reserve and the Newlands Forest (*SI Appendix, Table S1*). In total, we collected 14 Fynbos and 6 Forest species (including one forest margin species, labeled as forest [edge] in *SI Appendix, Table S1*). Detailed sampling techniques can be found in refs. 1 and 22.

We focused our analysis on the most distal first- and second-order roots. We derived the diameter of each root segment using SmartRoot (<https://smartroot.github.io>) to examine the high-resolution root scans (800 dots per inch). Each data point in Fig. 2A and *SI Appendix, Fig. S1A* represents the average diameter derived from the 5- to 10-point measurements along that root segment. For each species, we evaluated at least 20 individual root segments. Since we could not fully characterize the topological relationship between each examined first-order root and their parent second-order roots, we could also calculate the branching intensity and branching ratio for each plant species. Branching intensity measures the number of first-order root segments per length of second-order roots, while branching ratio measures the average number of first-order root segments per number of second-order roots. We calculated specific root length (Fig. 2C and *SI Appendix, Fig. S1A*) based on the standard formula $SRL = 4/(\pi * RTD * D^2)$, where root diameter (D) is measured in the field and root tissue density (RTD) is assumed to be 0.29 g/cm³ (global median from ref. 1). Reports of field-derived RTD from thin-rooted species are rare and highly uncertain due to the large volumes of soil that must be excavated to acquire sufficient root tissue. As a conservative approach, we therefore based our SRL calculations on the global median RTD for both Fynbos and Forest.

Nutrient Manipulation Experiment. We selected pristine Fynbos communities in two different sites, Swartboskloof and Langrivier, in the Jonkershoek Nature Reserve. Both sites feature a hot dry season and cool wet season, with annual rainfall ranging from 1,600 to 2,500 mm (53) and concentrated rainfall from May to September. Swartboskloof (33°57'S, 18°55'E) is a midelevation (415-m) flat plain (54). It is dominated by the tall shrubs *Protea neriifolia* and *Brunia nodiflora*. The Langrivier site (33°59'S, 18°58'E; 350 m) is similarly a flat

plain dominated by the taller *Protea repens*, and *P. neriifolia* shrubs (55). Both sites are perched on Table Mountain Group sandstones and have fairly deep soil (>30 cm), and the Langrivier site is comparatively clay-rich. Both sites were cleared by an intensive wildfire 2 mo before we started seedling transplantation.

Species Selection for Manipulation. We selected three tree species representative of a typical Afrotemperate Forest: *Kiggelaria africana*, *Diospyros whyteana*, and *Cassine peragua*. We excluded any riparian species (for example, the locally abundant *Cunonia capensis*) to minimize the effect of water on the survival and growth of transplanted seedlings (56). We also did not include indigenous nitrogen-fixing species [e.g., *Virgilia divaricata* (57)] in order to precisely evaluate the effect of nutrient manipulations. In their native habitats, our three species differ in their innate growth rates, drought resistances, and maximal mature heights. We believe they are representative of local Afrotemperate Forest patches, which are composed of some 10 to 30 tree species at a given forest patch (less diverse than a 10-m² Fynbos patch). We sourced these species from nearby nurseries.

Manipulation Design and Data Collection. We established 40 plots across the two sites (20 in each site) and manipulated nitrogen and phosphorus in a full factorial design (control, +N, +P, and +NP). Each nutrient treatment was replicated 10 times (5 replicates in each site), and its design (spatial configuration and randomization) is detailed in *SI Appendix, Fig. S2*. We planted six forest seedlings in each plot, two from each species, with one seedling in open soil and a second seedling inside a 30-cm-wide, 30-cm-deep polyvinyl chloride (PVC) collar to remove belowground resource competition (23). We randomized the location of seedlings to minimize potential uncontrolled errors due to underlying gradients in conditions (*SI Appendix, Fig. S3*).

To reduce the potential influence of “transplant shock” (58) caused by the sudden change of environment, we transplanted seedlings in the middle of the cold wet season, July 2015, about 1 mo before the first fertilization. This allowed seedlings enough time to acclimate. All roots were carefully rinsed to remove any legacy soil. We used wire cages 40 cm in diameter and 80 cm in height to control for the potential effect of local herbivores (e.g., *Otomys irroratus*).

We applied 60 kg·ha⁻¹·y⁻¹ of nitrogen to our nitrogen plots (+N and +NP), in the form of a combination of slow-releasing urea (40 kg·ha⁻¹·y⁻¹) and fast-releasing ammonium nitrate (20 kg·ha⁻¹·y⁻¹). We applied 5 kg/ha of phosphorus to our phosphorus plots (+P and +NP), in the form of triple phosphate (Ca₃(PO₄)₂). These addition rates were selected to increase both soil N and P at a stoichiometric ratio of 12:1 but also to not cause inhibitory effects on plant growth (43, 44, 59). Each year, we split the fertilization into two sessions: once in August to September toward the end of the rainy season, and once in March toward the end of the dry season. Tree seedlings were only watered during the first dry season in the first year of the experiment.

We periodically measured the diameter and height of tree seedlings by revisiting a permanent mark at the base of the seedling stem. We collected six measurements for each individual over ~4 y, with the first measurement in August 2015 and the last measurement in March 2019. For the local Fynbos community, the quick early-successional turnover and bushy configuration of plants made it impossible to consistently measure basal diameter. Instead, we used NDVI to quantify the growth of the Fynbos community. For this, we took multiband aerial photos using a Parrot Sequoia multispectral camera attached to a drone (DJI Phantom 3 Professional) during the rainy seasons of 2017 and 2019. Aerial photos were processed using Pix4Dmapper to generate NDVI maps, which were calibrated using a standard reflectance card. We georeferenced the NDVI maps to prominent land features of each site (e.g., big rocks, trails) before extracting the NDVI information of all experimental plots. We quantified Fynbos height by averaging across height measures taken at seven fixed loci (middle points between two adjacent seedlings; *SI Appendix, Fig. S3*), and revisited these measures in 2016 and 2019.

Data Compilation. We compiled data on ecosystem properties and aboveground plant traits from both Fynbos and Forest plant species in natural

communities, from the literature or our own unpublished data (Table 1). For soil organic carbon and soil nutrients, we compiled data from our own finely resolved study (15) of the Orange Kloof site in the Table Mountain National Park of Cape Town. At this site, both Fynbos and Forest biomes persist side-by-side on a deep base of granite underlying a top layer of Table Mountain Group quartzite (60). We identified five transects perpendicular to Fynbos–Forest boundaries and collected soils in July 2015 from 0- to 30-cm depth. We sampled three replicates within each community from each transect and measured total soil carbon, total soil nitrogen, and soil inorganic nutrients, including available phosphorus (citric acid method).

Statistical Analysis. We analyzed the trait differences between species (Fig. 2A and *SI Appendix, Fig. S1A*) and between biomes (Fig. 2 B–D and *SI Appendix, Fig. S1 B and C*) using linear regression (lm; R package stats) followed by pairwise contrast analysis (emmeans; R package emmeans). We log₁₀-transformed diameter, specific root length, length, branching intensity, and branching ratio across all analyses, consistent with the logarithmic scale of the y axis in Fig. 2 and *SI Appendix, Fig. S1*. We used a linear mixed-effects model (lmer; R package lme4) to evaluate whether specific root length differs between highly seasonal and nonforested biomes (Fynbos, Grassland, Desert, and Mediterranean) vs. less seasonal forested biomes (Tropical, Subtropical, Temperate, Southern Afrotemperate, and Boreal forests). With seasonality as the main effect, we treated species as a random effect nested within biome to account for unbalanced sample size within each biome (Fig. 2C). We compared the variation of branching ratio among three different biomes using Levene’s test of variance (leveneTest; R package car).

We analyzed seedling growth data using a linear mixed-effects model (lmer; R package lme4) with relative annual growth rate as the response variable; nutrient treatment and resource competition exclusion as fixed effects; site, replicate, plot, and individual plant as nested random effects; and species identity as a cross-random effect to control for the inherent differences between groupings. Relative growth rate is calculated as $\ln(D_1/D_0)/\Delta T$, where D_0 is the seedling basal diameter at the starting point, D_1 is the basal diameter at the ending point, and ΔT is the time interval between starting and ending points in years. We included only seedlings that were alive throughout the 4-y period of the experiment (2015 to 2019). We also analyzed Fynbos NDVI and height response to nutrient treatments using a linear mixed-effects model. For NDVI, we treated treatment as a fixed effect, and site and replicate as nested random effects. For Fynbos height, we treated treatment and time as fixed effects, and site, replicate, plot, and loci as nested random effects.

We derived *P* values in a linear mixed-effects model by comparing models with progressively simplified fixed effects (anova; R package stats). All analyses were conducted in R [v3.6.2 (61)].

Data Availability. Data reported in this article have been deposited in figshare (DOI: 10.6084/m9.figshare.17693747).

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